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Year: 2016

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DOI: <https://doi.org/10.1523/JNEUROSCI.1899-16.2016>

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ZORA URL: <https://doi.org/10.5167/uzh-128804>

Journal Article

Published Version

Originally published at:

Sherman, M A; LaCroix, M; Amar, F; Larson, M E; Forster, C; Aguzzi, A; Bennett, D A; Ramsden, M; Lesne, S E (2016). Soluble Conformers of A and Tau Alter Selective Proteins Governing Axonal Transport. *Journal of Neuroscience*, 36(37):9647-9658.

DOI: <https://doi.org/10.1523/JNEUROSCI.1899-16.2016>

# Soluble Conformers of A $\beta$ and Tau Alter Selective Proteins Governing Axonal Transport

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Despite the demonstration that amyloid- $\beta$  (A $\beta$ ) can trigger increased tau phosphorylation and neurofibrillary tangle (NFT) formation *in vivo*, the molecular link associating A $\beta$  and tau pathologies remains ill defined. Here, we observed that exposure of cultured primary neurons to A $\beta$  trimers isolated from brain tissue of subjects with Alzheimer's disease led to a specific conformational change of tau detected by the antibody Alz50. A similar association was supported by postmortem human brain analyses. To study the role of A $\beta$  trimers *in vivo*, we created a novel bigenic Tg-A $\beta$ +Tau mouse line by crossing Tg2576 (Tg-A $\beta$ ) and rTg4510 (Tg-Tau) mice. Before neurodegeneration and amyloidosis, apparent A $\beta$  trimers were increased by  $\sim$ 2-fold in 3-month-old Tg-A $\beta$  and Tg-A $\beta$ +Tau mice compared with younger mice, whereas soluble monomeric A $\beta$  levels were unchanged. Under these conditions, the expression of soluble Alz50-tau conformers rose by  $\sim$ 2.2-fold in the forebrains of Tg-A $\beta$ +Tau mice compared with nontransgenic littermates. In parallel, APP accumulated intracellularly, suggestive of a putative dysfunction of anterograde axonal transport. We found that the protein abundance of the kinesin-1 light chain (KLC1) was reduced selectively *in vivo* and *in vitro* when soluble A $\beta$  trimers/Alz50-tau were present. Importantly, the reduction in KLC1 was prevented by the intraneuronal delivery of Alz50 antibodies. Collectively, our findings reveal that specific soluble conformers of A $\beta$  and tau cooperatively disrupt axonal transport independently from plaques and tangles. Finally, these results suggest that not all endogenous A $\beta$  oligomers trigger the same deleterious changes and that the role of each assembly should be considered separately.

**Key words:** Alzheimer's disease; amyloid-beta; axonal transport; brain; oligomer; tau

## Significance Statement

The mechanistic link between amyloid- $\beta$  (A $\beta$ ) and tau, the two major proteins composing the neuropathological lesions detected in brain tissue of Alzheimer's disease subjects, remains unclear. Here, we report that the trimeric A $\beta$  species induce a pathological modification of tau in cultured neurons and in bigenic mice expressing A $\beta$  and human tau. This linkage was also observed in postmortem brain tissue from subjects with mild cognitive impairment, when A $\beta$  trimers are abundant. Further, this modification of tau was associated with the intracellular accumulation of the precursor protein of A $\beta$ , APP, as a result of the selective decrease in kinesin light chain 1 expression. Our findings suggest that A $\beta$  trimers might cause axonal transport deficits in AD.

## Introduction

In our current understanding of the physiopathology of Alzheimer's disease (AD), the soluble forms of the amyloid- $\beta$  peptide

(A $\beta$ ) and the microtubule-associated protein tau have been proposed to be more important than the fibrillar aggregates that have classically characterized this disorder (Walsh et al., 2002; Cleary et al., 2005; Santacruz et al., 2005; Lesné et al., 2006; Berger et al., 2007; Roberson et al., 2007; Shankar et al., 2008). Despite the seminal demonstrations that A $\beta$  exposure can lead to increased

Received May 31, 2016; revised July 19, 2016; accepted July 31, 2016.

Author contributions: S.E.L. designed research; M.A.S., M.L., F.A., M.E.L., C.F., M.R., and S.E.L. performed research; A.A., D.A.B., and S.E.L. contributed unpublished reagents/analytic tools; M.A.S., M.L., F.A., M.E.L., M.R., and S.E.L. analyzed data; S.E.L. wrote the paper.

This work was supported by the National Institutes of Health (Grants R00AG031293 to S.E.L. and R01NS33249 to Karen H. Ashe and Grants P30AG10161 and R01AG15819 to D.A.B.) and the University of Minnesota Foundation (start-up funds to S.E.L.). We thank Karen Ashe for Tg2576 and rTg4510 mice and L. Kotilinek, L. Kemper, J. Starks, and J. Paulson for technical help.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.1899-16.2016

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tau phosphorylation and neurofibrillary tangle (NFT) formation in animals (Götz et al., 2001; Lewis et al., 2001; Oddo et al., 2003), the exact molecular mechanisms associating A $\beta$  and tau remain poorly understood (Attems et al., 2011; Larson and Lesné, 2012; Lesné, 2013).

Due to the inherent biology of neuronal cells, axonal transport is critical for neuronal function and survival. Multiple neurodegenerative disorders, including AD, present with alterations of fast axonal transport, which have been proposed to represent an early pathological event (Goldstein, 2001; Stokin et al., 2005; Ittner et al., 2009; Morfini et al., 2009; Muresan and Muresan, 2009). Soluble assemblies of A $\beta$ , also called A $\beta$  oligomers (oA $\beta$ s), have been shown to be capable of inhibiting axonal transport in cultured cells (Rui et al., 2006; Pigino et al., 2009). Additional reports refined this concept by demonstrating that oligomeric mixtures of synthetic A $\beta$  disrupt axonal transport *in vitro* (Decker et al., 2010; Vossel et al., 2010; Vossel et al., 2015).

In addition to A $\beta$ , tau is known to be concentrated preferentially in axons, where it stabilizes microtubules that serve as tracks for the transport of organelles, vesicles, and proteins (Hirokawa and Takemura, 2005) and has been proposed to induce neuronal cell death by interfering with microtubule-dependent axonal transport (Stamer et al., 2002). Despite convincing observations showing that tau alters axonal transport *in vitro* (Ebner et al., 1998; Dixit et al., 2008), it is less clear whether tau acts similarly *in vivo* (Yuan et al., 2008). Recent studies indicated that, although tau did not appear to affect axonal transport under baseline conditions, tau protein levels were critical for axonal transport in the presence of synthetic A $\beta$  oligomers (Vossel et al., 2010).

While assessing the effects of purified forms of endogenous oA $\beta$ s on tau posttranslational modifications, we found that AD-brain-derived A $\beta$  trimers applied onto primary neurons at single-digit nanomolar concentrations induced a selective conformation change of tau detected by the antibody Alz50 (Carmel et al., 1996). Supporting this *in vitro* finding, we found that protein levels of A $\beta$  trimers, described previously to peak in the brain tissues of Religious Orders Study (ROS) participants with mild cognitive impairment (MCI) (Lesné et al., 2013), were positively correlated with soluble Alz50-tau levels. Upon characterizing the newly created bigenic Tg-A $\beta$ +Tau mouse model overexpressing the human APP and human tau, we observed that soluble A $\beta$  trimers increased independently of monomeric A $\beta$  levels before neurodegeneration and amyloidosis in the forebrains of these mice. In association with the rise in A $\beta$  trimers observed in young bigenic mice, soluble Alz50-positive tau levels were also elevated, whereas other pathological forms of tau were not. In parallel, APP accumulated intracellularly in brain tissue of bigenic mice, suggesting possible axonal transport defects. When analyzing putative modulations in the abundance of proteins governing axonal transport, the protein expression of the light chain of kinesin-1 (KLC1) was lowered markedly, whereas other motor proteins appeared to be unaffected. To evaluate the potential effects of A $\beta$  trimers on proteins regulating axonal transport, we exposed primary cultured neurons to purified A $\beta$  species. These conditions recapitulated the selective changes in KLC1 observed *in vivo*, indicating that A $\beta$  trimers are a potent disruptor of KLC1 expression in neurons. Finally, we showed that this effect was not dependent on the expression of the cellular form of the prion protein PrP<sup>C</sup>, but did require the presence of tau gene expression and Alz50-tau conformers.

## Materials and Methods

### Transgenic mice

Mice from the APP line Tg2576 (MGI\_2385631), which express the human APP with the Swedish mutation (APP<sup>KM670/671NL</sup>) directed by the hamster prion protein promoter (Hsiao et al., 1996), were crossed with rTg4510 mice (MGI\_4819866) (Ramsden et al., 2005; Santacruz et al., 2005) overexpressing the P301L mutant of four-repeat tau lacking the N-terminal sequences 4R0N (tau<sup>P301L</sup>). Both lines were kindly provided by Dr. Karen Ashe (University of Minnesota). Briefly, Tg4510 responders (FVB/N) were mated with CKTTA activators (129S6) to generate FVB129S6F1-rTg4510 mice. rTg4510<sup>+/+</sup> mice were subsequently mated with Tg2576 (129S6) to generate mixed-background mice (129S6)FVB129S6F1-rTg4510xTg2576<sup>+/+/+</sup>. Dr. Adriano Aguzzi (University Hospital of Zurich, Switzerland) kindly provided *Prnp*-null mice (MGI\_1888773) (Büeler et al., 1992). Htau (MGI\_3057129) (Andorfer et al., 2003) and *Mapt*-null mice (Tucker et al., 2001) were purchased from Jackson Laboratories. All experiments described here were conducted in full accordance with Association for Assessment and Accreditation of Lab Animal Care and Institutional Animal Care and Use Committee guidelines, with every effort made to minimize the number of mice used. Both male and female mice were used in all experiments. Experimenters were kept blinded to the genotype of the animal groups until raw data were obtained.

### Primary cell cultures

Mouse cortical cultures of neurons were prepared from 14- to 15 d-old embryos as described previously (Lesné et al., 2005; Larson et al., 2012) using  $5 \times 10^5$  cells/dish. After 3 d *in vitro* (DIV), neurons were treated with 10  $\mu$ M cytosine  $\beta$ -D-arabinofuranoside (AraC) to inhibit proliferation of non-neuronal cells. All experiments were performed on nearly pure neuronal cultures (>98% of microtubule associated protein-2 immunoreactive cells) after 12–14 DIV. Six to eight 35 mm dishes per culture per condition were used across three independent experiments.

### Protein extractions

For analyzing A $\beta$  species, two extractions protocols described previously were used (Lesné et al., 2006; Shankar et al., 2008; Sherman and Lesné, 2011). In particular, membrane-enriched protein extracts (MB extracts) refer to protein lysates obtained after the third step of a serial extraction with a lysis RIPA buffer comprised of 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, 3% SDS, and 1% deoxycholate. As detailed in a methodology chapter published recently (Sherman and Lesné, 2011), samples were then centrifuged at 16,100  $\times$  g for 90 min. Supernatants were collected and pellets further extracted with formic acid to analyze fibrillar/deposited proteins. It is possible that the use of the RIPA lysis buffer might strip loosely bound A $\beta$  from plaques.

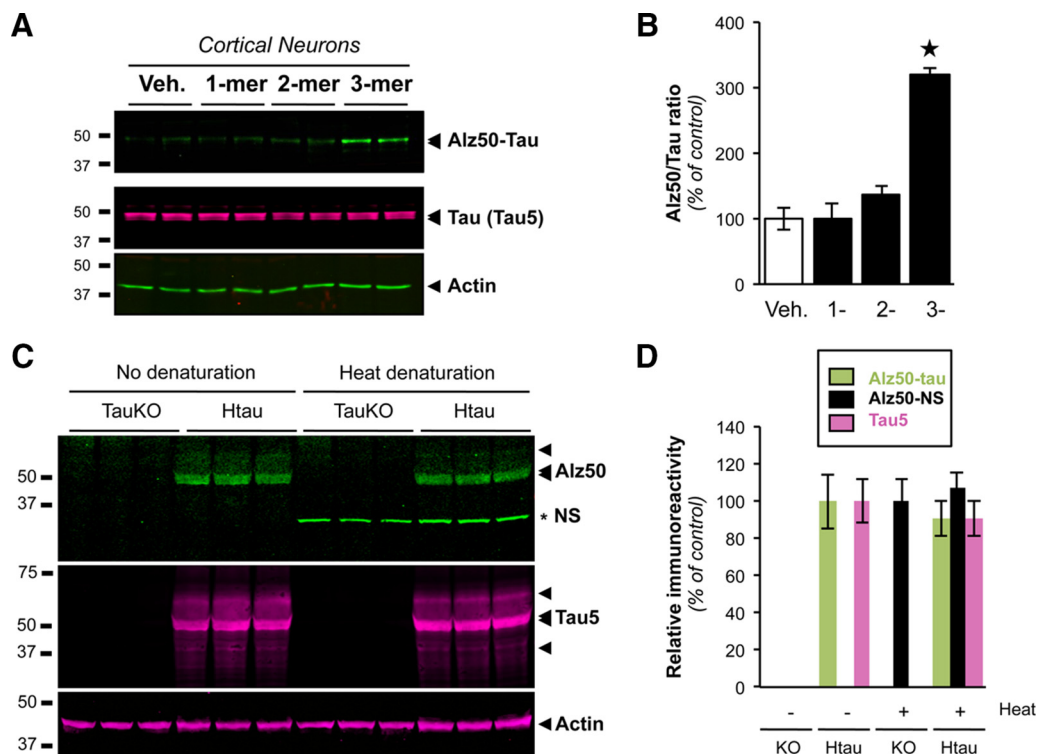
Protein amounts were determined by the Bradford protein assay (BCA Protein Assay, Pierce). All supernatants were ultracentrifuged for 60 min at 100,000  $\times$  g. Finally, before analysis, fractions with endogenous immunoglobulins were depleted by incubating extracts sequentially for 1 h at room temperature with 50  $\mu$ l of Protein A-Sepharose, Fast Flow followed by 50  $\mu$ l of Protein G-Sepharose, Fast Flow (GE Healthcare Life Sciences).

### Purification of human A $\beta$ oligomers from AD brain tissue

Human brain tissue came from participants in the ROS who died with MCI. Details of the study have been described previously (Bennett et al., 2012). The study was approved by the Institutional Review Board or Rush University Medical Center and all subjects signed informed consent and an Anatomic Gift Act. Soluble oligomeric A $\beta$  species were purified from AD brain tissue, as reported previously by our group (Larson et al., 2012). Relative amounts of purified oligomeric A $\beta$  were calculated based on synthetic A $\beta$ <sub>1–42</sub> standards (0.001, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.5 ng; Sigma-Aldrich) run alongside the samples used for experiments.

### Western blotting and quantification

**SDS-PAGE.** Electrophoreses were done on precast 10–20% polyacrylamide Tris-Tricine gels and 10.5–14% and 4–10.5% Tris-HCl gels (Bio-Rad). Protein levels were normalized to 2–100  $\mu$ g of protein per sample



**Figure 1.** Application of soluble A $\beta$  species purified from AD brain tissues induces selective tau pathological changes in mouse primary cortical neurons. **A**, Protein levels of tau conformers detected with Alz50 (Alz50-Tau) and total tau as assessed by Western blot using Alz50 and Tau5 antibodies in wild-type primary neurons exposed to human-brain-purified oligomeric A $\beta$  (1 nM for 1 h). Actin was used as an internal control. **B**, Quantitation of the modified tau/total tau ratios across conditions revealed a significant conformational change of tau after exposure to A $\beta$  trimers (lanes 7 and 8). This effect was not detected in neurons treated with soluble A $\beta$  monomers or dimers. Error bars indicate mean  $\pm$  SD, ANOVA ( $F_{(3,25)} = 281.604, p < 0.0001$ ) followed by Student's *t* test,  $\star p < 0.05, n = 6-8/\text{treatment}$ ). **C**, **D**, Influence of sample denaturation on Alz50 immunoreactivity using Western blotting (**C**, **D**). Alz50-NS corresponds to the nonspecific  $\sim 25$  kDa band detected by Alz50. **D**, After quantitation of the Western blot data, two-way ANOVA ( $F_{(3,23)} = 57.2621, p < 0.0001$ ) revealed a significant effect of the genotype ( $F_{(1,23)} = 170.766, p < 0.0001$ ), but no effect of the treatment ( $F_{(1,23)} = 1.017, p = 0.3427$ ) and no genotype $\times$ treatment interaction ( $F_{(1,23)} = 0.002, p = 0.961$ ). Error bars indicate the mean  $\pm$  SD;  $n = 6/\text{genotype/condition}$ .

(depending on targeted protein) and resuspended with 4 $\times$  Tricine loading buffer before boiling (5 min at 95°C with agitation at 1250 rpm).

**Transfer.** Thereafter, proteins were transferred to 0.2  $\mu\text{m}$  nitrocellulose membrane (Bio-Rad).

**Blotting.** Nitrocellulose membranes were boiled in 50 ml of PBS by microwaving for 25 and 15 s with a 3 min interval. Membranes were blocked in TTBS (Tris-buffered saline plus 0.1% Tween 20) containing 5% bovine serum albumin (BSA) (Sigma-Aldrich) for 1–2 h at room temperature, and probed with appropriate antisera/antibodies diluted in 5% BSA-TTBS. Primary antibodies were probed either with anti-IgG immunoglobulins conjugated with biotin or InfraRed dyes (Li-Cor Biosciences). When biotin-conjugated secondary antibodies were used, IR-conjugated Neutravidin (Pierce) was added to amplify the signal. Blots were revealed on an Odyssey platform (Li-Cor Biosciences).

**Stripping.** When required, membranes were stripped using Restore Plus Stripping buffer (Pierce) for 30–180 min at room temperature depending on antibody affinity.

**Quantification.** Densitometry analyses were performed using Opti-Quant (Packard Bioscience) or Odyssey (Li-Cor) software. Each protein of interest was probed in three individual experiments under the same conditions and quantified by software analysis after determination of experimental conditions ascertaining linearity in the detection of the signal and are expressed as density light units (DLUs). The method used allows for a dynamic range of  $\sim 100$ -fold above background ( $0.01 \times 10^6$  DLUs). Respective averages were then determined across the triplicate Western blots. Normalization was performed against the neuron-specific nuclear protein NeuN, also performed in triplicate. None of the protein brain levels measured correlated with postmortem interval, arguing against potential protein degradation within human tissues (data not shown).

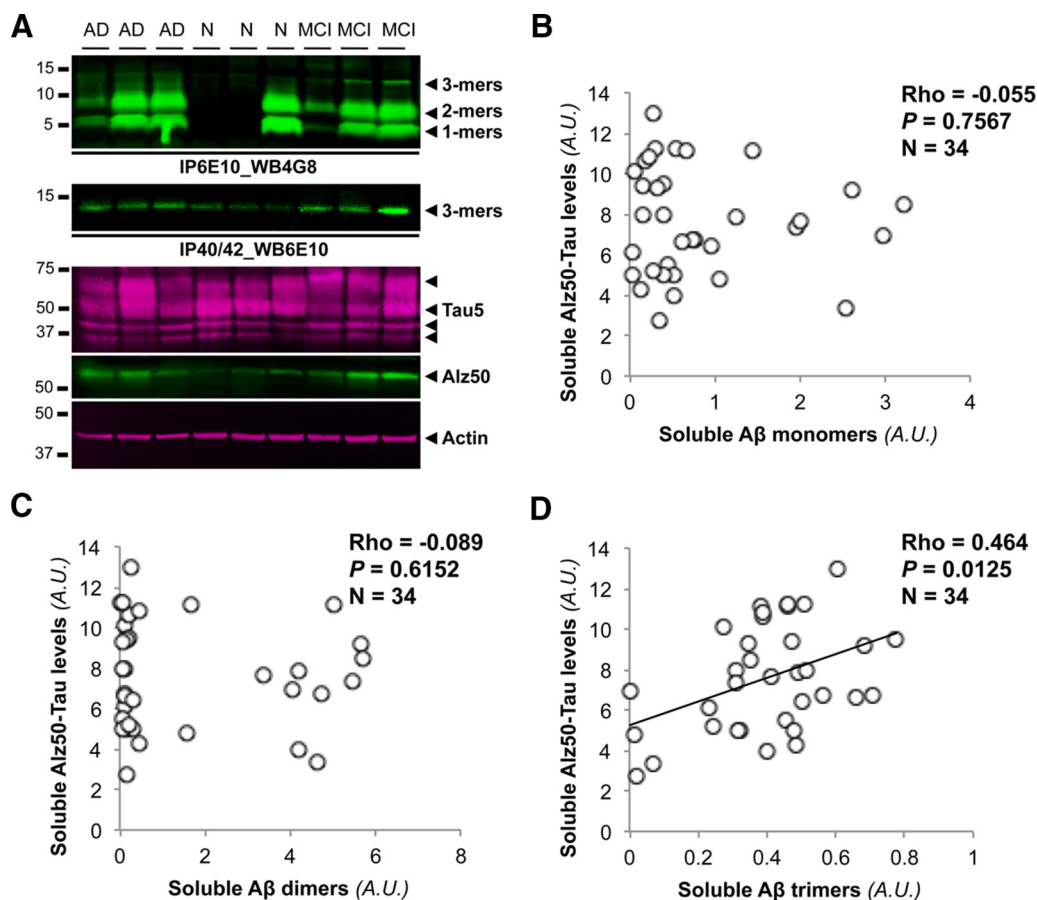
### Immunoprecipitations

Aliquots (200  $\mu\text{g}$ ) of protein extracts were diluted to 1 ml with dilution buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl) and incubated with the appropriate antibodies (5  $\mu\text{g}$  of 6E10 or Mab2.1.3/13.1.1 antibodies) overnight at 4°C. Then, 50  $\mu\text{l}$  of Protein G-Sepharose, Fast Flow (GE Life Sciences) 1:1 (v:v) slurry solution with dilution buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, pH 7.4) was added for 2 h. The beads were washed twice in 1 ml of dilution buffer and proteins eluted in 25  $\mu\text{l}$  of loading SDS-PAGE buffer by boiling.

### Antibodies

The following primary antibodies were used in this study: 6E10 (1:2500; BioLegend catalog #803003, RRID: AB\_10175145) and 4G8 (1:2500; BioLegend catalog #800703, RRID: AB\_662812), biotinylated-6E10 (1:2500; BioLegend catalog #803009, RRID: AB\_10175146), 22C11 (1:2000; Millipore catalog #MAB348, RRID: AB\_94882), Tau5 (1:2000; BioLegend catalog #806403, RRID: AB\_10175718), Alz50 (1:2000; kind gifts from Peter Davies, Albert Einstein College of Medicine), 40-/42-end specific Mab2.1.3 and Mab13.1.1 (1:1000; kind gifts from Pritam Das, Mayo Jacksonville), anti-NeuN (1:5000; Millipore catalog #MAB377B, RRID: AB\_177621), anti-MAP2 (1:500; Novus catalog #NB300–213, RRID: AB\_2138178), anti-KLC-1 (1:1000; Millipore catalog #MAB1616, RRID: AB\_94286 and Santa Cruz Biotechnology catalog #sc-25735, RRID: AB\_2280879), anti-kinesin superfamily protein 5 (anti-KIF-5) (1:1000; Abcam catalog #ab62104, RRID: AB\_2249625), anti-JNK-interacting protein 1 (anti-JIP-1) (1:1000; Abcam catalog #ab24449, RRID: AB\_448056 and Abcam catalog #ab78948, RRID: AB\_1640605), anti-Dynein (1:2000; Abcam catalog #ab75214, RRID: AB\_1280872), anti-EB-3 (1:2000; Abcam catalog #ab99287, RRID: AB\_10676513), anti-Actin (1:10,000; Millipore catalog #MAB1501, RRID: AB\_2223041 and Sigma-Aldrich catalog #A2066, RRID:





**Figure 2.** Soluble Aβ trimers levels are positively correlated to soluble Alz50-tau conformers in the inferior temporal gyrus of individuals diagnosed with MCI in the ROS cohort. **A**, Representative Western blot images for Aβ and tau proteins in a subset of ROS study participants (9 out of 84). Aβ levels were measured following immunoprecipitation using 6E10 (top insert) or a mixture of 40- and 42-end specific antibodies (middle insert). 4G8, 6E10, Tau5, and Alz50 antibodies were used for Western blotting detection. Actin was used as control. **B–D**, Regression analyses between low-molecular-weight soluble Aβ species and soluble Alz50-tau conformers in ROS MCI measured (Spearman's rho correlation,  $n = 34$ ). The arbitrary units for Aβ and tau protein levels are on the same scale and correspond to  $\times 10^6$  densitometry light units, as reported in Lesné et al. (2013).

AB\_476693), and anti- $\alpha$ -tubulin (1:100,000; Sigma-Aldrich catalog #T6074, RRID:AB\_477582).

### Statistical analyses

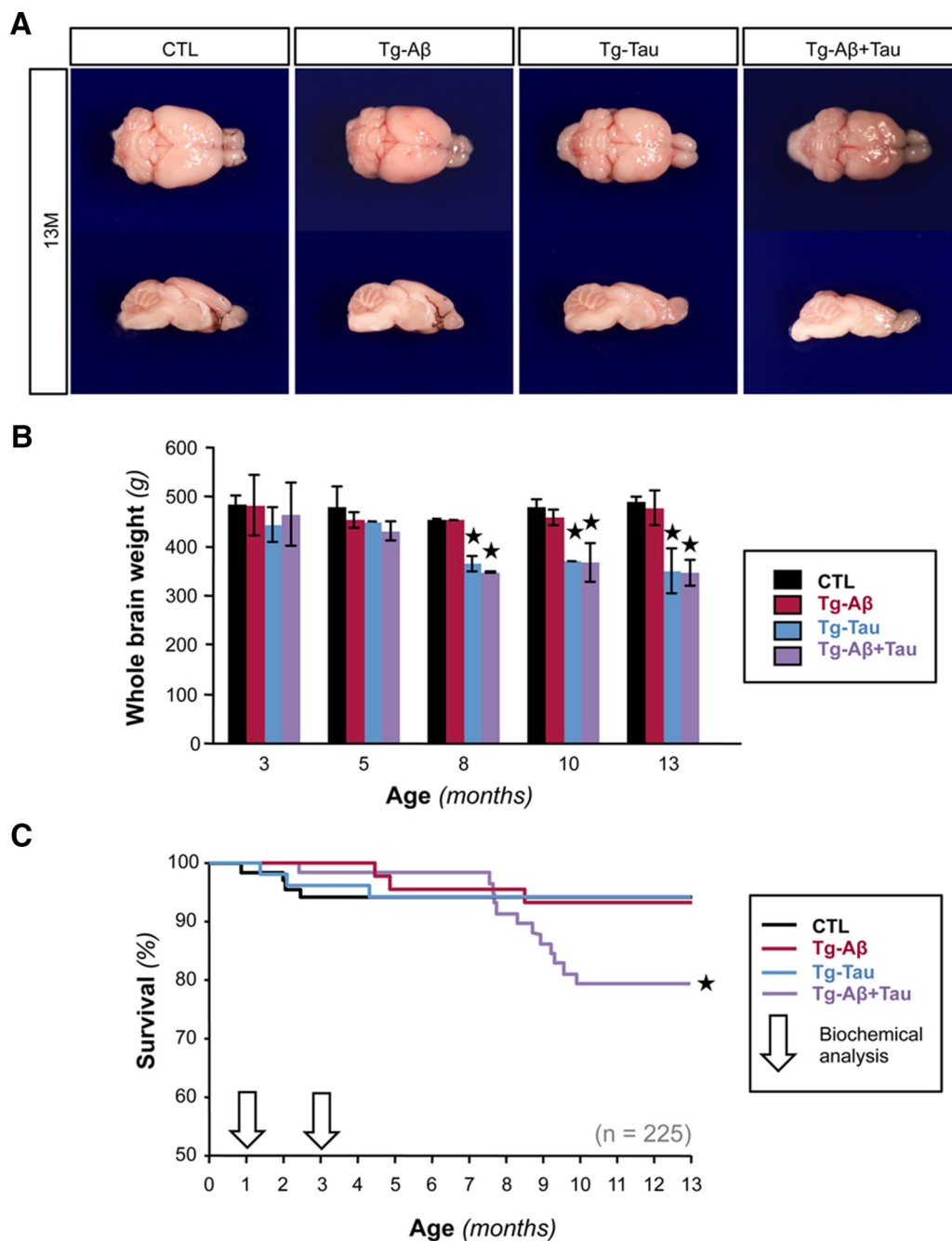
When variables were non-normally distributed, nonparametric statistics were used (Spearman's rho correlation coefficients, Kruskal–Wallis nonparametric ANOVA followed by Bonferroni-corrected two-group *post hoc* Mann–Whitney U tests). When variables were normally distributed, the following parametric statistics were used (one/two-way ANOVA followed by Bonferroni-corrected two-group *post hoc* Student's *t* tests). Sample size was determined by power analysis to be able to detect statistically significant changes within a 20% variation of measured responses. Analyses were performed using JMP 11 or JMP12 (SAS Institute).

## Results

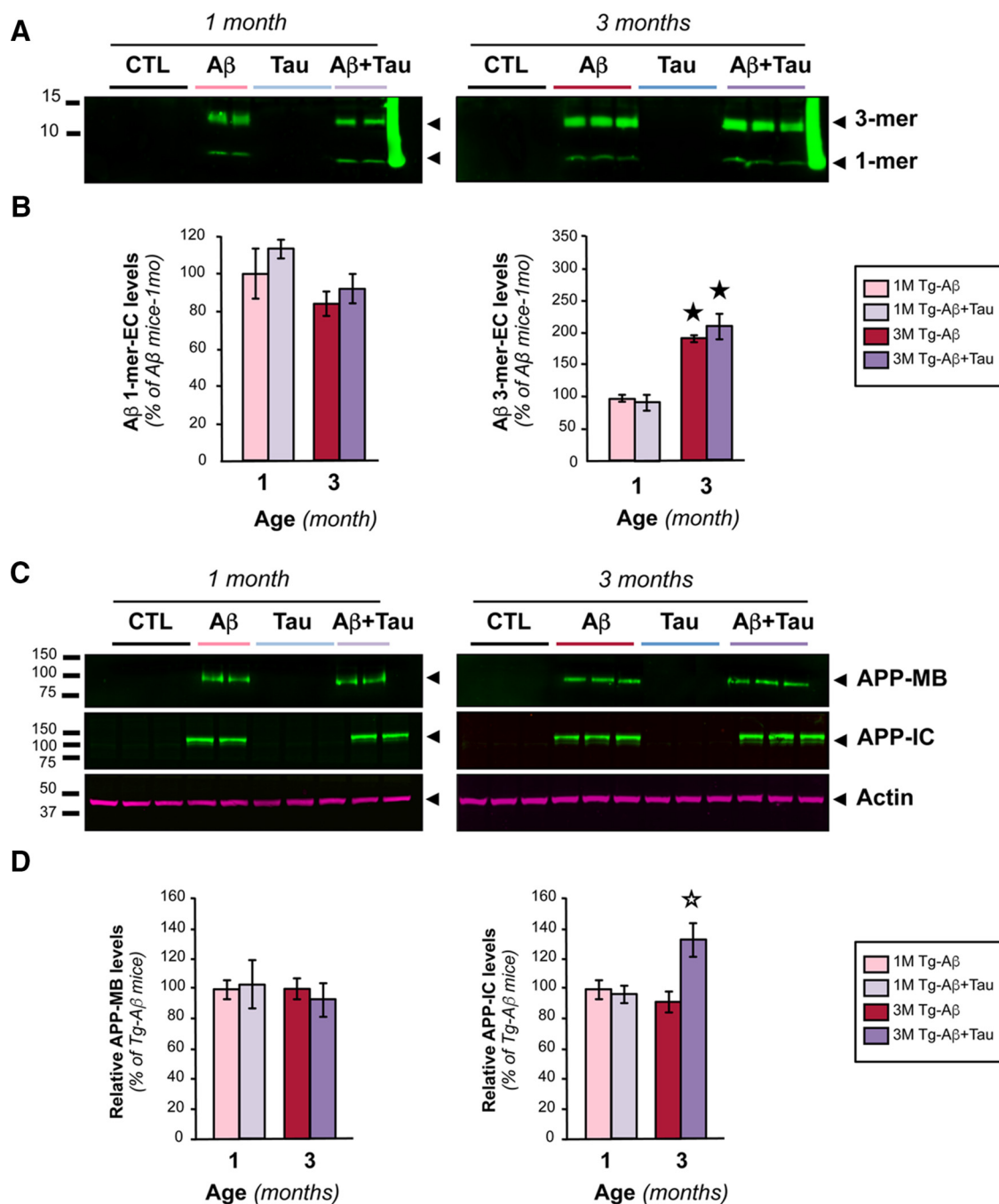
### Endogenous Aβ trimers induce distinct tau pathological changes *in vitro*

We reported previously that Aβ dimers and trimers purified from AD brain tissue applied at low nanomolar concentrations induce the hyperphosphorylation of tau at tyrosine 18 (Y18) mediated by the Src kinase Fyn in mouse cortical neurons (Larson et al., 2012). To determine whether other disease-relevant tau modifications were triggered under these conditions by these low-molecular-weight Aβ oligomers, we used a panel of well characterized antibodies, including CP13, PHF1, PG5, and Alz50, that detect either phosphorylation at serine residues 202 (S202),

396/404 (S396/404), and 409 (S409) or aberrant misfolding of tau, respectively (Fig. 1) Although tau was hyperphosphorylated at Y18 by purified Aβ dimers and trimers, no apparent enhanced phosphorylation at S202, S396/404, and S409 was observed compared with cells treated with vehicle or monomeric Aβ (data not shown). However, we observed a 3.2-fold increase in Alz50 immunoreactivity in neurons exposed to Aβ trimers compared with control cells (Fig. 1A,B). Applying equimolar concentrations of monomeric or dimeric Aβ did not trigger such a change. Because the epitope for Alz50 was reported to be sensitive to denaturation (Carmel et al., 1996), we compared the detection of Alz50-tau molecules in 12-month-old Htau mice (Andorfer et al., 2003) and *MAPT*<sup>−/−</sup> mice (Tucker et al., 2001). Half of the samples were denatured by boiling before loading onto PAGE gels and the other half were not subjected to heat denaturation. Using Western blotting, we did not observe differences in the ability to detect Alz50-tau in Htau mice due to sample denaturation (Fig. 1C,D). The sample denaturation step led to the detection of a prominent nonspecific  $\sim 25$  kDa band by Alz50 antibodies in all specimens, including age-matched mouse *MAPT*<sup>−/−</sup> littermates (Fig. 1C). These results would therefore argue that heat denaturation does not prevent the accurate detection of Alz50-positive tau conformers by Western blot. Overall, these



**Figure 3.** Tissue atrophy and survival rates in Tg-Tau + Aβ mice. **A**, Representative images show brain tissue from 13-month-old mice (top, aerial view; bottom, cross-sectional view). **B**, Mean weights of whole-brain tissue binned by age. Tg-tau and Tg-Aβ + Tau values were statistically different from both Tg-con and Tg-Aβ. Two-way ANOVA ( $F_{(18, 138)} = 27.5016$ ,  $p < 0.0001$ ) revealed a significant effect of age ( $F_{(4, 138)} = 39.077$ ,  $p < 0.0001$ ), genotype ( $F_{(3, 138)} = 68.396$ ,  $p < 0.001$ ), and a significant age\*transgene interaction ( $F_{(11, 138)} = 9.661$ ,  $p < 0.0001$ ,  $\star p < 0.0001$  vs control). **C**, Kaplan–Meier survival curves showing effect of the overexpression of P301L-tau on premature mortality in Tg-Aβ mice. Despite the development of substantial pathology in both Tg2576 and rTg4510 mice, neither transgenic line experienced a significant increase in mortality. In control mice ( $n = 69$ ), we detected an approximate 5% decrease in the population within the first 400 d. This represented an expected minority of mice that died spontaneously or were killed due to colony-associated ailments or injury. This was also the case in Tg-Aβ and Tg-Tau mice despite the development of Aβ plaque and neurofibrillary tangle pathologies, respectively. In fact, we recorded little further mortality in these lines as the mice continued to age past 400 d (Tg-Aβ 91% survival at 767 d,  $n = 45$ , 4 deaths; Tg-Tau 94% survival at 603 d,  $n = 53$ , 3 deaths). In Tg-Aβ + Tau mice, a similar rate of premature death occurred up to the age of ~230 d (96% survival at 236 d,  $n = 58$ , 2 deaths). However, after 230 d, we observed a sudden increase in the numbers of spontaneous deaths such that survival rates decreased to 79% of expected control levels. Interestingly, increased death occurred up to the age of ~300 d, after which survival rates plateaued and further loss of Tg-Aβ + Tau mice was not recorded. Decreased survival between 230 and 300 d was a consistent phenomenon that was observed across multiple independent cohorts of aging mice and was not provoked by a change in housing conditions. Sex appeared to influence vulnerability to premature death in Tg-Aβ + Tau mice because 3 females (25%,  $n = 29$ ) and 9 males (75%,  $n = 29$ ) accounted for those found dead in the home cage. Because Tg-Aβ + Tau mice that lived to be >300 d of age had a moribund phenotype and were extremely inactive, all remaining +/+ mice assigned to the current study were killed at ~400 d. All genotyped mice in the colony were included in the analysis ( $N = 251$ ). By log-rank comparison, only Tg-Aβ + Tau mice differed from all other groups ( $\chi^2_{(3)} = 19.629$ ,  $\star p = 0.0002$  vs control).



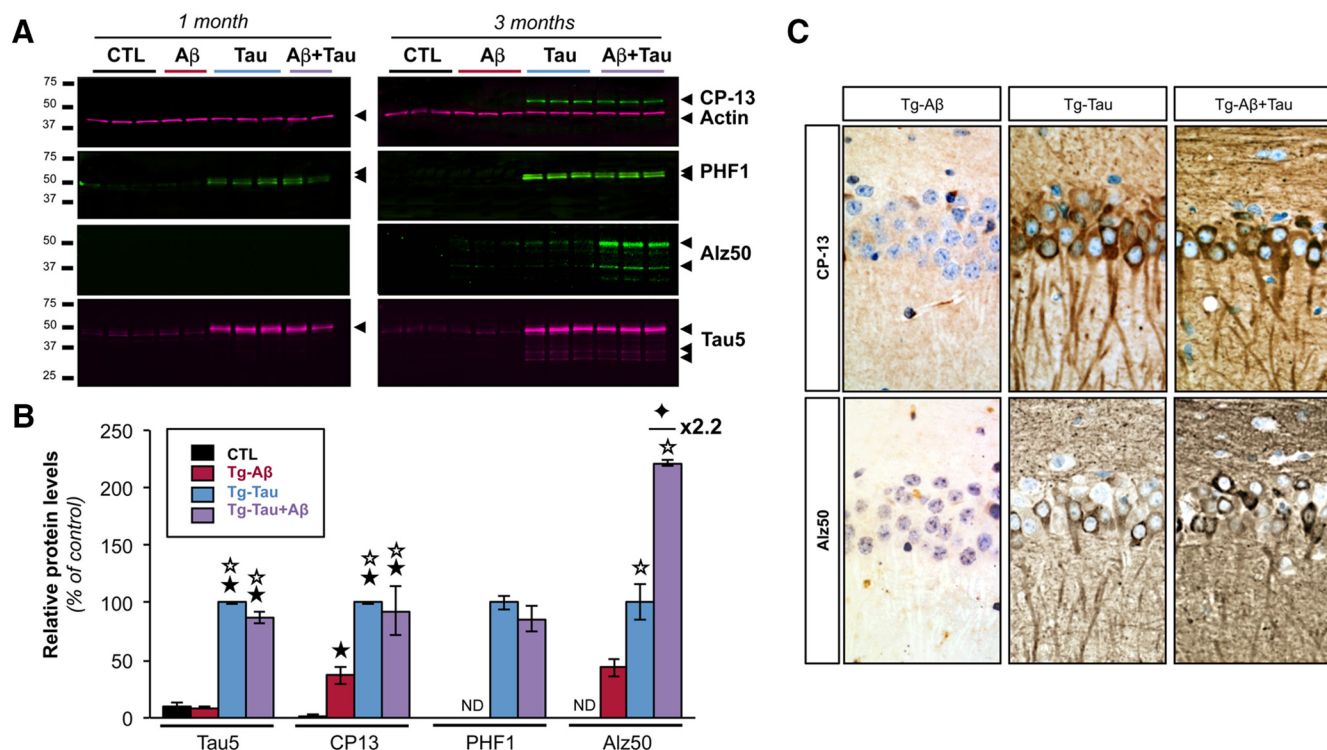
**Figure 4.** Age-dependent expression of soluble A $\beta$  and APP species in forebrain tissues of bigenic Tg-A $\beta$  + Tau mice. **A**, Representative images of soluble A $\beta$  detected in nontransgenic (control), Tg-A $\beta$ , Tg-Tau, and Tg-A $\beta$  + Tau mice by immunoprecipitation with 40/42-end-specific A $\beta$  antibodies and revealed by Western blot using 6E10. **B**, Quantitation of the levels of soluble A $\beta$  species across genotypes revealed an ~2-fold elevation of A $\beta$  trimers in 3-month-old mice expressing hA $\beta$  compared with younger littermates. No differences were observed between Tg-A $\beta$  and Tg-A $\beta$  + Tau mice. Error bars indicate the mean  $\pm$  SD. Two-way ANOVA ( $F_{(3,26)} = 106.623, p < 0.0001$ ) revealed a significant effect of age ( $F_{(1,26)} = 305.939, p < 0.0001$ ), but no effect of the genotype ( $F_{(1,26)} = 1.305, p = 0.2651$ ) and no significant age\*transgene interaction ( $F_{(1,26)} = 1.848, p = 0.1871$ ) (\* $p < 0.05$  vs 1-month-old A $\beta$  mice,  $n = 5$ –7/age/genotype). **C**, Representative images of full-length human APP detected in either membrane-enriched (top insert) or intracellular-enriched (bottom insert) fractions of nontransgenic (control), Tg-A $\beta$ , Tg-Tau, and Tg-A $\beta$  + Tau mice using 6E10. **D**, Densitometry analyses revealed an ~31% accumulation of intracellular APP in 3-month-old Tg-A $\beta$  + Tau mice compared with age-matched Tg-A $\beta$  mice. Error bars indicate the mean  $\pm$  SD. ANOVA (two-way ANOVA,  $F_{(3,26)} = 13.719, p < 0.0001$ ) revealed a significant effect of age ( $F_{(1,26)} = 16.599, p < 0.0001$ ), genotype ( $F_{(1,26)} = 12.778, p = 0.0016$ ), and a significant age\*transgene interaction ( $F_{(1,26)} = 7.358, p = 0.0124$ ) (\* $p < 0.05$  vs 1-month-old A $\beta$  mice,  $\star p < 0.05$  vs 1-month-old A $\beta$  mice,  $n = 5$ –7/age/genotype).

findings suggested that A $\beta$  trimers induce tau misfolding selectively.

#### Brain levels of A $\beta$ trimers correlate with soluble Alz50-tau in human tissue

Previously, we reported that apparent A $\beta$  trimers are elevated in ROS participants with MCI compared with age-matched con-

trols and subjects with AD (Lesné et al., 2013). We therefore examined whether the abundance of trimeric A $\beta$  species was correlated with the levels of soluble Alz50-tau conformations in intracellular-enriched fractions of the inferior temporal gyrus of our MCI cohort (Fig. 2A). We observed that neither soluble monomeric nor dimeric A $\beta$  levels were related to Alz50-tau levels (Fig. 2B,C). In contrast, we found a strong positive correlation



**Figure 5.** Age-dependent expression of tau species in forebrain tissues of bigenic Tg-Aβ + Tau mice. **A**, Western blot analyses of soluble tau detected in control, Tg-Aβ, Tg-Tau, and Tg-Aβ + Tau mice using CP13, PHF1, Alz50, and Tau5 antibodies displayed apparent changes in Alz50-Tau in brain tissue of bigenic mice. **B**, Quantitation of the levels of soluble tau species across genotypes revealed an ~2.2-fold elevation of Alz50-positive tau conformers in 3-month-old mice Tg-Aβ + Tau compared with Tg-Tau mice. Error bars indicate the mean ± SD. ANOVA ( $F_{\text{Tau5}}^{(3, 28)} = 578.315, p < 0.0001$ ;  $F_{\text{CP13}}^{(3, 28)} = 242.249, p < 0.0001$ ;  $F_{\text{PHF1}}^{(3, 28)} = 156.129, p < 0.0001$ ;  $F_{\text{Alz50}}^{(3, 28)} = 371.269, p < 0.0001$ , respectively) followed by Student's *t* test (\* $p < 0.05$  vs 3-month-old control mice, <sup>†</sup> $p < 0.05$  vs 3-month-old Tg-Aβ mice, <sup>♦</sup> $p < 0.05$  vs 3-month-old Tg-Tau mice,  $n = 5$ –7/age/genotype). **C**, Although no obvious change was observed for CP13-Tau between Tg-Tau and Tg-Aβ + Tau mice by immunohistochemical analysis, changes in soluble Alz50-tau species within CA1 pyramidal neurons of Tg-Aβ + Tau mice were obvious at 3 months.

(Spearman's rho = 0.464,  $p = 0.0125$ ,  $n = 34$ ) between Aβ trimers measured in extracellular-enriched fractions and soluble Alz50-tau species (Fig. 2D). This observation suggested that the findings obtained *in vitro* could be pathophysiologically relevant in the context of AD.

### Elevated levels of Aβ trimers in young Tg-Aβ + Tau mice

To attempt to create *in vivo* experimental conditions in which Aβ trimers would be elevated and that would allow us to study the relationship between Aβ and tau, we generated novel Tg-Aβ + Tau mice by crossing Tg2576 mice (Tg-Aβ) with rTg4510 mice (Tg-Tau). We chose these lines for the following reasons: (1) the brain tissue of Tg2576 mice displays relatively high levels of extracellular Aβ trimers in 1- to 3-month-old transgenic mice (Lesné et al., 2006); (2) both Tg2576 and the CKTTA activator line were in a 129S6 background strain, thereby minimizing genetic background mixing; (3) plaque deposition and tangle formation occur at ~9 months in Tg-Aβ and at ~4.5 months in Tg-Tau mice, respectively (Hsiao et al., 1996; Santacruz et al., 2005), allowing a fairly wide temporal window to analyze the effects of soluble forms of Aβ and tau independently of deposited amyloids; and (5) tau-induced neurodegeneration is observed at ~5 months of age in Tg-Tau mice, providing the opportunity to study the interaction of soluble human Aβ and tau before cell loss (Ramsden et al., 2005). Once lines were generated, we first assessed whether tissue atrophy in Tg-Aβ + Tau mice was altered compared with that documented for Tg-Tau mice. Measuring whole-brain weights revealed no apparent differences between Tg-Tau and Tg-Aβ + Tau groups at any of the ages studied (3, 5, 8, 10, and 13 months; Fig. 3A,B). However, survival analyses in-

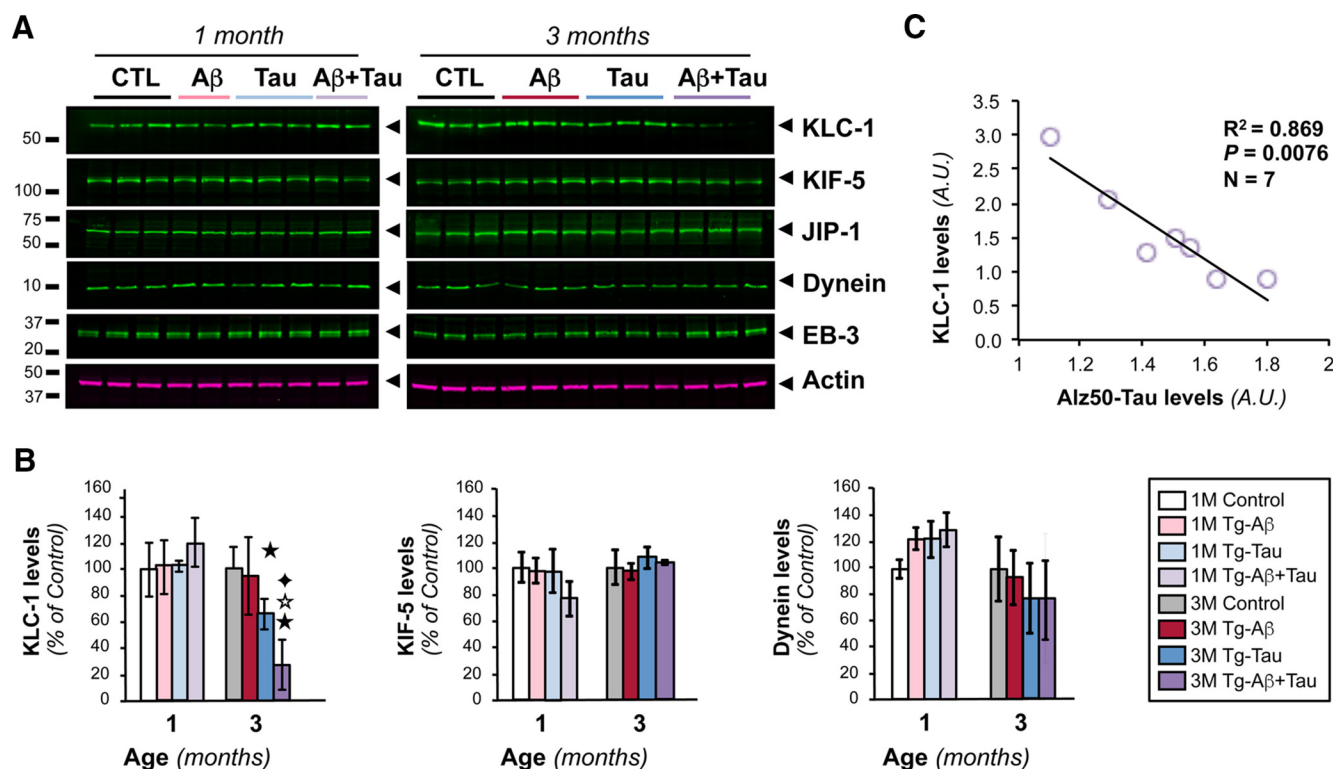
dicated that Tg-Aβ + Tau mice displayed enhanced lethality starting at ~8 months of age (Fig. 3C). Because Tg-Aβ mice display relatively high levels of extracellular Aβ trimers in 3-month-old mice (Lesné et al., 2006), we measured the expression of Aβ and its precursor protein APP across genotypes at 1 and 3 months of age (Fig. 4). Using immunoprecipitations with 40/42-end specific antibodies of Aβ, we found that brain levels of Aβ monomers were similar at both ages in extracellular-enriched fractions of Tg-Aβ and Tg-Aβ + Tau mice (Fig. 4A,B). The abundance of trimers Aβ rose by ~2-fold between at 1 and 3 months in APP-overexpressing mice, a 1.92- and 2.09-fold increase, respectively, in 3-month-old Tg-Aβ and Tg-Aβ + Tau brains (Fig. 4B).

Protein expression of full-length human APP pools was assessed in membrane-associated extracts and intracellular-enriched extracts by Western blotting using 6E10 (Fig. 4C). The pool of APP bound to membranes was similar between Tg-Aβ and Tg-Aβ + Tau mice (Fig. 4C,D). However, we observed a  $30.58 \pm 7.97\%$  increase in APP levels in intracellular enriched lysates, whereas actin protein levels used as an internal control were unchanged (Fig. 4C,D). Similar results were obtained using aminoterminal (22C11) or carboxyterminal (APPCTer-C17) antibodies against APP (data not shown), consistent with an intracellular accumulation of full-length APP.

### Rise in Aβ trimers is associated with distinct conformational tau changes *in vivo*

Given that brain levels of apparent Aβ trimers increased by 2-fold without modifying soluble monomeric Aβ levels in 3-month-old Tg-Aβ + Tau mice, this experimental condition allowed us to test





**Figure 6.** Selective lowering of KLC-1 in bigenic Tg-Aβ+Tau mice. **A, B**, Western blot analyses of proteins governing axonal transport using brain tissue of nontransgenic (control), Tg-Aβ, Tg-Tau, and Tg-Aβ+Tau mice revealed a downregulation of KLC1 in Tg-Tau mice overexpressing human tau, which was enhanced in Tg-Aβ+Tau bigenic mice (an ~2.4-fold potentiation of the effect observed) at 3 months of age. Error bars indicate the mean ± SD. Two-way ANOVA ( $F_{KLC1}^{(7, 49)} = 16.972, p < 0.0001$ ) revealed a significant effect of age ( $F_{KLC1}^{(1, 49)} = 24.229, p < 0.0001$ ), genotype ( $F_{KLC1}^{(3, 49)} = 15.256, p < 0.0001$ ), and a significant age\*transgene interaction ( $F_{KLC1}^{(3, 49)} = 14.893, p < 0.0001$ ), \* $p < 0.05$  vs 3-month-old Aβ mice,  $\star p < 0.05$  vs 3-month-old Aβ mice,  $\blacklozenge p < 0.05$  vs 3-month-old Tau mice,  $n = 5$ –7/age/genotype). **C**, Regression analysis between KLC1 protein levels and soluble Alz50-tau conformers in 3-month-old Tg-Aβ+Tau ( $F_{(1, 5)} = 24.7712, p = 0.0076, n = 7$ ).

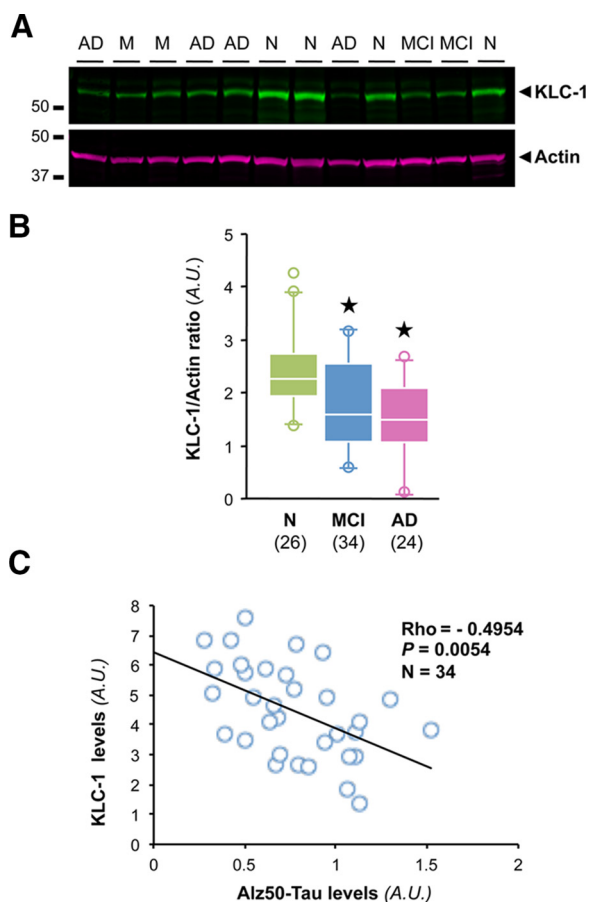
whether soluble Alz50-tau molecules were exclusively augmented in this environment (Fig. 5). We first compared putative modulations in tau hyperphosphorylation and conformation observed in the early or late stages of neurodegenerative disorders involving tau. Western blotting analyses of intracellular extracts revealed no changes in CP13-Tau or PHF1-Tau levels between Tg-Tau and Tg-Aβ+Tau mice (Fig. 5A,B), indicating that Aβ was not potentiating these disease-related tau changes. However, we detected a 2.2-fold elevation of soluble tau conformers detected with Alz50 in bigenic Tg-Aβ+Tau mice compared with Tg-Tau littermates (Fig. 5A,B). Importantly, total tau levels measured with Tau5 antibodies remained unchanged across Tg-Tau and Tg-Aβ+Tau mice, suggesting that the conformation of tau was altered in the presence of constant expression. To support these biochemical analyses of tau, we performed immunohistochemical studies on brain sections from Tg-Aβ, Tg-Tau and Tg-Aβ+Tau mice using CP13 and Alz50 antibodies (Fig. 5C). Although CP13-Tau immunoreactivity within the CA1 hippocampal neurons appeared comparable between Tg-Tau and Tg-Aβ+Tau mice (Fig. 5C, top row), a clear increase in Alz50 staining was readily observed in the neuronal soma of CA1 pyramidal neurons of bigenic mice compared with Tg-Tau mice (Fig. 5C, bottom row). Overall, the 2-fold elevation of Aβ trimers in 3-month-old Tg-Aβ+Tau mice is associated with a selective ~2-fold increase in tau misfolding.

#### Twofold elevation in Aβ trimers/Alz50-tau is associated with selective deficits in KLC1 governing anterograde axonal transport

Because tau is critical for axonal transport in the presence of synthetic Aβ oligomers *in vitro* (Vossel et al., 2010) and APP

appeared to accumulate intracellularly in 3-month-old Tg-Aβ+Tau mice, we hypothesized that the accumulation of soluble Alz50-tau molecules associated with the elevation of Aβ trimers in bigenic mice could alter the proteins governing anterograde axonal transport. We therefore assessed the expression levels of motor proteins responsible for anterograde axonal transport, KLC-1, KIF-5, and the scaffold protein JIP-1, and for retrograde axonal transport, dynein. At 1 month of age, the abundance of the measured proteins appeared unaffected (Fig. 6A,B). Two months later, the expression of KLC-1 was reduced to  $65.82 \pm 11.51\%$  in forebrain tissue of Tg-Tau mice compared with nontransgenic mice. Importantly, KLC-1 expression further dropped to ~28% in bigenic Tg-Aβ+Tau mice, a 2.4-fold decrease compared with Tg-Tau mice (Bonferroni-corrected *t* test after ANOVA,  $p = 0.0012, n = 5$ –6/age/genotype). Not all proteins regulating anterograde axonal transport were impaired because neither KIF-5 nor JIP-1 protein expression changed across genotypes. In addition, the brain levels of the motor protein dynein did not seem to be modified at the ages and genotype tested (ANOVA,  $p > 0.05, n = 5$ –6/age/genotype). We next assessed whether KLC-1 expression levels were related to those of Alz50-tau species in bigenic Tg-Aβ+Tau mice and found a negative correlation between these two variables (Fig. 6C). These data suggest the possibility that the increases in Alz50-tau and Aβ trimers observed in bigenic Tg-Aβ+Tau mice might trigger this change in KLC-1 expression.

To determine whether a similar association was observed in human brains, we measured KLC-1 protein expression in the ROS cohort (Fig. 7A). The relative expression of KLC-1 was reduced in the AD group, consistent with earlier reports (Mo-



**Figure 7.** Relationship between KLC-1 and Alz50-tau species in MCI brains. **A**, Western blot analysis of KLC-1 protein levels in the ROS cohort analyzed. Actin was used as an internal standard. The images shown only correspond to a subset of the specimens studied (12 of 84). **B**, Box plot of the relative levels of KLC-1 across clinical groups. Italicized numbers in parentheses indicate group sizes. No cognitive impairment (“N”) is shown in green, MCI is shown in blue, and AD is indicated by pink boxes. The bar inside the box indicates the median; the upper and lower limits of boxes represent the 75<sup>th</sup> and 25<sup>th</sup> percentiles, respectively; and bars flanking the box represent the 95<sup>th</sup> and fifth percentiles (\* $p < 0.05$  vs N, Kruskal–Wallis test ( $\chi^2_{(2)} = 13.499$ ,  $p = 0.0012$ ), followed by Mann–Whitney  $U$  test,  $N = 84$ ). **C**, Regression analyses between KLC1 protein levels and soluble Alz50-tau conformers in ROS MCI measured (Spearman’s rho correlation,  $n = 34$ ).

rel et al., 2012), and in the MCI group (Fig. 7B). Because trimeric A $\beta$  levels are highest in MCI subjects from the ROS cohort (Lesné et al., 2013), we examined the relationship between KLC-1 and Alz50-tau in this group. In this context, the abundance in KLC-1 protein was inversely correlated with tau conformers detected by Alz50 (Fig. 7C).

### A $\beta$ trimers trigger decreases in KLC-1 protein mediated by tau conformers

To evaluate directly the potential of A $\beta$  trimers to lower KLC-1 protein levels, we treated mouse cortical neurons with either brain-derived A $\beta$  trimers (1–2 nM for 60 min) or vehicle and examined KLC-1 and KIF-5 expression in these cultured primary cells (Fig. 8A,B). Upon treatment with A $\beta$  trimers, KLC-1 expression was remarkably reduced to  $47.42 \pm 5.64\%$  compared with control neurons ( $p < 0.001$ ,  $n = 6$ –8/treatment). Consistent with the *in vivo* findings reported above, no changes in KIF-5 protein expression were observed. Because PrP<sup>C</sup> and tau have been proposed to mediate oA $\beta$ -induced toxicity (Roberson et al., 2007; Laurén et al., 2009; Vossel et al., 2010; Larson et al., 2012;

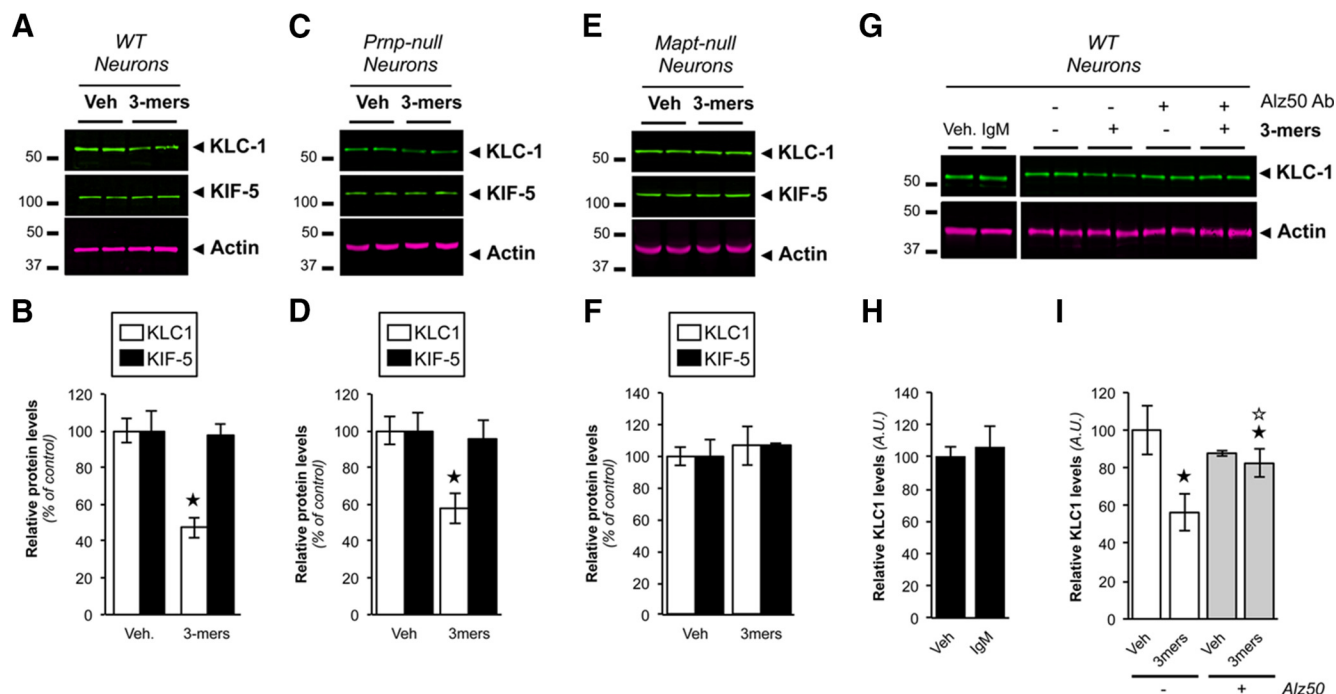
Um et al., 2012), we next assessed whether PrP<sup>C</sup> (Fig. 8C,D) or tau gene products (Fig. 8E,F) were mediating the lowering of KLC1 induced by A $\beta$  trimer exposure in *Prnp*-null or *Mapt*-null cortical neurons. In agreement with our previous study indicating that A $\beta$  dimers, but not A $\beta$  trimers, were coimmunoprecipitating with PrP<sup>C</sup> (Larson et al., 2012), deletion of the gene encoding for PrP<sup>C</sup> did not rescue the decrease in KLC-1 proteins levels when A $\beta$  trimers were applied to cells (Fig. 8C,D). Similarly to WT neurons, KIF-5 expression was unaltered in *Prnp*-null neurons (Fig. 8D).

To determine whether changes in tau induced by AD brain-purified A $\beta$  trimers preceded KLC-1 reductions, we applied A $\beta$  trimers or vehicle onto *Mapt*-null cortical neurons (Fig. 8E,F). Contrary to the 50–55% reduction in KLC-1 observed in WT or *Prnp*-null neurons, KLC-1 protein levels were unaffected by A $\beta$  trimers in tau-deficient neurons. This result demonstrated that A $\beta$ -induced deficits in KLC-1 required expression of tau. Finally, to establish that soluble Alz50-tau conformers were necessary to mediate the selective decrease in KLC-1 triggered by trimeric A $\beta$  application, we preconditioned neurons by delivering Alz50 or control IgM antibodies intracellularly using Chariot technology 30 min before exposure to A $\beta$  trimers (Fig. 8G–I). Intraneuronal delivery of control immunoglobulins did not alter KLC-1 expression (Fig. 8G,H), arguing against a possible nonspecific effect of antibody delivery. When the Chariot shuttling reagent was applied alone, application of A $\beta$  trimers led to an ~44% lowering in KLC-1, similar to what was observed in naive WT neurons (Fig. 8A,B). In contrast, Alz50-pretreated neurons appeared to be protected from the effect of A $\beta$  trimers on KLC-1 (Fig. 7H). These findings established directly that Alz50-positive conformers were required to alter KLC-1 protein levels in the presence of A $\beta$  trimers.

## Discussion

Despite accumulating evidence supporting the concept that oligomeric forms of A $\beta$  constitute the biological vessel responsible for synaptic dysfunction in AD, the exact role of each assembly of A $\beta$  remains unknown and shrouded in controversy (Benilova et al., 2012; Lesné, 2013). Our group recently suggested that A $\beta$  molecules identified as AD brain-tissue-purified A $\beta$  dimers and trimers by immunological, electrophoretic, and liquid-phase separation techniques activate the kinase Fyn similarly *in vitro* (Larson et al., 2012). In the same report, we proposed that the cellular form of the prion protein PrP<sup>C</sup> acted as a transducing receptor for A $\beta$  dimers, but not A $\beta$  trimers, thereby inducing the hyperphosphorylation of tau at Y18 (Larson et al., 2012). Importantly, neither soluble A $\beta$ \*56 nor protofibrillar A $\beta$ s nor A $\beta$  monomers triggered the phosphorylation of Fyn/tau under similar conditions, suggesting that not all A $\beta$  oligomers induce the same intracellular signaling pathways. This notion is particularly important in the context of earlier reports demonstrating that the respective abundance of apparent A $\beta$  dimers, trimers, and A $\beta$ \*56 varies across clinical groups in humans (Shankar et al., 2008; Lesné et al., 2013). One likely consequence of these studies is that each oligomeric A $\beta$  assembly might stimulate distinct cellular pathways during the course of the disease, all of which (maybe sequentially or synergistically) constitute the key molecular events underlying AD.

The hypothesis developed above led us to assess whether additional selective pathological changes of tau might occur in primary cortical neurons exposed to oA $\beta$  species purified from biologically relevant brain tissue. Surprisingly, we found that soluble Alz50-positive tau conformers were specifically induced



**Figure 8.** Trimeric A $\beta$ -induced selective lowering of KLC-1 is dependent on tau conformers in primary cortical neurons. Mouse primary cortical neurons were exposed to either vehicle or 1 nM trimeric A $\beta$  for 1 h. **A, B**, Protein levels of KLC1 were altered in cultured neurons after exposure with A $\beta$  trimers, whereas KIF-5 and actin were unchanged. Error bars indicate the mean  $\pm$  SD ( $t$  test,  $t_{(7.88)} = 9.476$ ,  $p < 0.0001$ ,  $\star p < 0.05$  vs vehicle,  $n = 6-8$ /treatment). **C, D**, In *Prnp*-null primary neurons, similar findings were observed to those described in **A** and **B**. Error bars indicate the mean  $\pm$  SD ( $t$  test ( $t_{(8)} = 9.147$ ,  $p < 0.001$ ),  $\star p < 0.05$  vs vehicle,  $n = 8$ /treatment). **E, F**, In *Mapt*-null neurons, KLC1 protein levels were not downregulated when A $\beta$  trimers were applied to cells. Error bars indicate the mean  $\pm$  SD ( $t$  test,  $\star p < 0.05$  vs vehicle,  $n = 6-8$ /treatment). **G–I**, Effect of pretreatments with IgM (**H**) or Alz50 (**I**) delivered using Chariot delivery technology on KLC1 deficits triggered by A $\beta$  trimers. Error bars indicate the mean  $\pm$  SD ( $t$  test,  $t_{(14)} = 0.548$ ,  $p > 0.05$ ,  $\star p < 0.05$  vs vehicle,  $n = 6-8$ /treatment; ANOVA,  $F_{(3,25)} = 63.227$ ,  $p < 0.0001$ , followed by Student's  $t$  test,  $\star p < 0.05$  vs vehicle,  $\star p < 0.05$  vs A $\beta$  3-mers,  $n = 6-8$ /treatment for **H** and **I**, respectively).

upon treatment with A $\beta$  trimers. The Alz50 antibody is a monoclonal antibody that was initially found to stain fibrillar tau pathology in AD brain tissue (Wolozin et al., 1986; Wolozin and Davies, 1987). Its epitope was subsequently identified to recognize a folded conformation of tau containing amino acids 2–10 and 312–342 (Carmel et al., 1996; Kopeikina et al., 2013). This folding change was not detected in cells exposed to other soluble forms of A $\beta$  tested here (i.e., A $\beta$  monomers, dimers, A $\beta$ \*56, and A $\beta$  protofibrils). This finding led us to question whether similar changes could be observed *in vivo* when A $\beta$  trimers are abundant. Based on our prior characterization of the Tg2576 mouse line (Lesné et al., 2006; Lesné et al., 2008), we anticipated that crossing Tg2576 with rTg4510 mice would allow us to studying a potential interaction between A $\beta$  trimers and tau pathological changes. In very young bigenic mice at 3 months of age, in which forebrain levels of apparent trimeric A $\beta$  were doubled, we indeed observed a selective change in tau detected by Alz50. Perhaps not coincidentally, it is interesting to us that the amplitude of the change in the elevation of A $\beta$  trimers between 1- and 3-month-old mice (a 2.09-fold increase) is similar to the amplitude in the change of soluble tau conformers detected by Alz50 (a 2.2-fold increase). In parallel, full-length APP seemed to accumulate in the intracellular-enriched protein fractions of Tg-A $\beta$ +Tau mice, suggesting that APP trafficking might be compromised. Because synthetic preparations of A $\beta$  oligomers can alter tau-mediated axonal transport *in vitro* (Vossel et al., 2010), we tested whether increases in these soluble forms of A $\beta$  and tau were associated with modulations in the expression of proteins regulating axonal transport. Consistent with previous observations indicating that alterations of fast axonal transport represent an early pathological event (Stokin et al., 2005;

Muresan and Muresan, 2009), KLC-1 protein levels, which were already impaired in Tg-Tau brains, were further reduced in Tg-A $\beta$ +Tau, whereas KIF-5, JIP-1, and Dynein protein levels appeared to be unaltered. This observation suggested that A $\beta$  trimers/Alz50-tau might cooperate in selectively altering KLC-1-dependent mechanisms. To demonstrate that A $\beta$  trimers were the initiator molecule in that cascade, we treated cultured neurons with A $\beta$  trimers purified from AD brain tissue and validated that neuronal exposure with this oligomeric assembly was sufficient to reduce KLC-1 expression selectively. Although other groups have reported that uncharacterized synthetic mixtures of A $\beta$  oligomers alter tau-mediated axonal defects (Vossel et al., 2010), we believe that our *in vitro* and *in vivo* findings are the first to demonstrate that a specific endogenous oligomeric A $\beta$  assembly alters proteins governing axonal transport. To our knowledge, this report also constitutes the first description of selective conformational changes in tau induced by different A $\beta$  oligomers. Finally, taking into consideration that brain levels of A $\beta$  trimers appear to be highest in subjects with MCI and lower in AD (Lesné et al., 2013), these results highlight the importance of considering the impact of each A $\beta$  oligomer individually. Indeed, we predict that a strategy interfering with A $\beta$  trimers/Alz50-tau would only prevent axonal transport alterations during the prodromal stage of AD. Based on this current work and earlier reports suggesting that different oA $\beta$ s trigger distinct pathological changes during disease progression (Larson et al., 2012; Lesné et al., 2013), we believe that separate therapies aimed at disrupting specific oA $\beta$ /tau interactions will need to be considered to treat patients at the preclinical AD, MCI, or AD stages.



## References

- Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, Duff K, Davies P (2003) Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem* 86:582–590. [CrossRef Medline](#)
- Attens J, Ittner A, Jellinger K, Nitsch RM, Maj M, Wagner L, Götz J, Heikenwalder M (2011) Reduced secretogin expression in the hippocampus of P301L tau transgenic mice. *J Neural Transm* 118:737–745. [CrossRef Medline](#)
- Benilova I, Karran E, De Strooper B (2012) The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat Neurosci* 15:349–357. [CrossRef Medline](#)
- Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS (2012) Overview and findings from the religious orders study. *Curr Alzheimer Res* 9:628–645. [CrossRef Medline](#)
- Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, Wszolek Z, Ashe K, Knight J, Dickson D, Andorfer C, Rosenberry TL, Lewis J, Hutton M, Janus C (2007) Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *J Neurosci* 27:3650–3662. [CrossRef Medline](#)
- Büeler H, Fischer M, Lang Y, Bluethmann H, Lipp HP, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C (1992) Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* 356:577–582. [CrossRef Medline](#)
- Carmel G, Mager EM, Binder LI, Kuret J (1996) The structural basis of monoclonal antibody Alz50's selectivity for Alzheimer's disease pathology. *J Biol Chem* 271:32789–32795. [CrossRef Medline](#)
- Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci* 8:79–84. [CrossRef Medline](#)
- Decker H, Lo KY, Unger SM, Ferreira ST, Silverman MA (2010) Amyloid-beta peptide oligomers disrupt axonal transport through an NMDA receptor-dependent mechanism that is mediated by glycogen synthase kinase 3beta in primary cultured hippocampal neurons. *J Neurosci* 30:9166–9171. [CrossRef Medline](#)
- Dixit R, Ross JL, Goldman YE, Holzbaur EL (2008) Differential regulation of dynein and kinesin motor proteins by tau. *Science* 319:1086–1089. [CrossRef Medline](#)
- Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E (1998) Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol* 143:777–794. [CrossRef Medline](#)
- Goldstein LS (2001) Kinesin molecular motors: transport pathways, receptors, and human disease. *Proc Natl Acad Sci U S A* 98:6999–7003. [CrossRef Medline](#)
- Götz J, Chen F, van Dorpe J, Nitsch RM (2001) Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Abeta 42 fibrils. *Science* 293:1491–1495. [CrossRef Medline](#)
- Hirokawa N, Takemura R (2005) Molecular motors and mechanisms of directional transport in neurons. *Nat Rev Neurosci* 6:201–214. [CrossRef Medline](#)
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102. [CrossRef Medline](#)
- Ittner LM, Ke YD, Götz J (2009) Phosphorylated Tau interacts with c-Jun N-terminal kinase-interacting protein 1 (JIP1) in Alzheimer disease. *J Biol Chem* 284:20909–20916. [CrossRef Medline](#)
- Kopeikina KJ, Polydoro M, Tai HC, Yaeger E, Carlson GA, Pitstick R, Hyman BT, Spire-Jones TL (2013) Synaptic alterations in the rTg4510 mouse model of tauopathy. *J Comp Neurol* 521:1334–1353. [CrossRef Medline](#)
- Larson ME, Lesné SE (2012) Soluble Abeta oligomer production and toxicity. *J Neurochem* 120:125–139. [Medline](#)
- Larson M, Sherman MA, Amar F, Nuvoletone M, Schneider JA, Bennett DA, Aguzzi A, Lesné SE (2012) The complex PrP(c)-Fyn couples human oligomeric Abeta with pathological tau changes in Alzheimer's disease. *J Neurosci* 32:16857–16871a. [CrossRef Medline](#)
- Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457:1128–1132. [CrossRef Medline](#)
- Lesné SE (2013) Breaking the code of amyloid-oligomers. *Int J Cell Biol* 2013:950783. [CrossRef Medline](#)
- Lesné SE, Sherman MA, Grant M, Kuskowski M, Schneider JA, Bennett DA, Ashe KH (2013) Brain amyloid-beta oligomers in ageing and Alzheimer's disease. *Brain* 136:1383–1398. [CrossRef Medline](#)
- Lesné S, Ali C, Gabriel C, Croci N, MacKenzie ET, Glabe CG, Plotkine M, Marchand-Verrecchia C, Vivien D, Buisson A (2005) NMDA receptor activation inhibits alpha-secretase and promotes neuronal amyloid-beta production. *J Neurosci* 25:9367–9377. [CrossRef Medline](#)
- Lesné S, Koh MT, Kotilinek L, Kaye R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 440:352–357. [CrossRef Medline](#)
- Lesné S, Kotilinek L, Ashe KH (2008) Plaque-bearing mice with reduced levels of oligomeric amyloid-beta assemblies have intact memory function. *Neuroscience* 151:745–749. [CrossRef Medline](#)
- Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 293:1487–1491. [CrossRef Medline](#)
- Morel M, Héraud C, Nicaise C, Suain V, Brion JP (2012) Levels of kinesin light chain and dynein intermediate chain are reduced in the frontal cortex in Alzheimer's disease: implications for axoplasmic transport. *Acta Neuropathol* 123:71–84. [CrossRef Medline](#)
- Morfini GA, Burns M, Binder LI, Kanaan NM, LaPointe N, Bosco DA, Brown RH Jr, Brown H, Tiwari A, Hayward L, Edgar J, Nave KA, Garberrn J, Atagi Y, Song Y, Pignio G, Brady ST (2009) Axonal transport defects in neurodegenerative diseases. *J Neurosci* 29:12776–12786. [CrossRef Medline](#)
- Muresan V, Muresan Z (2009) Is abnormal axonal transport a cause, a contributing factor or a consequence of the neuronal pathology in Alzheimer's disease? *Future Neurol* 4:761–773. [CrossRef Medline](#)
- Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 39:409–421. [CrossRef Medline](#)
- Pignio G, Morfini G, Atagi Y, Deshpande A, Yu C, Jungbauer L, LaDu M, Busciglio J, Brady S (2009) Disruption of fast axonal transport is a pathogenic mechanism for intraneuronal amyloid beta. *Proc Natl Acad Sci U S A* 106:5907–5912. [CrossRef Medline](#)
- Ramsden M, Kotilinek L, Forster C, Paulson J, McGowan E, SantaCruz K, Guimaraes A, Yue M, Lewis J, Carlson G, Hutton M, Ashe KH (2005) Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L). *J Neurosci* 25:10637–10647. [CrossRef Medline](#)
- Roberson ED, Searce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L (2007) Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 316:750–754. [CrossRef Medline](#)
- Rui Y, Tiwari P, Xie Z, Zheng JQ (2006) Acute impairment of mitochondrial trafficking by beta-amyloid peptides in hippocampal neurons. *J Neurosci* 26:10480–10487. [CrossRef Medline](#)
- Santacruz K, Lewis J, Spire T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH (2005) Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 309:476–481. [CrossRef Medline](#)
- Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14:837–842. [CrossRef Medline](#)
- Sherman MA, Lesné SE (2011) Detecting abeta\*56 oligomers in brain tissues. *Methods Mol Biol* 670:45–56. [CrossRef Medline](#)
- Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM (2002) Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J Cell Biol* 156:1051–1063. [CrossRef Medline](#)
- Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, Masliah E, Williams DS, Goldstein LS (2005) Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* 307:1282–1288. [CrossRef Medline](#)



- Tucker KL, Meyer M, Barde YA (2001) Neurotrophins are required for nerve growth during development. *Nat Neurosci* 4:29–37. [CrossRef Medline](#)
- Um JW, Nygaard HB, Heiss JK, Kostylev MA, Stagi M, Vortmeyer A, Wisniewski T, Gunther EC, Strittmatter SM (2012) Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat Neurosci* 15:1227–1235. [CrossRef Medline](#)
- Vossel KA, Zhang K, Brodbeck J, Daub AC, Sharma P, Finkbeiner S, Cui B, Mucke L (2010) Tau reduction prevents Abeta-induced defects in axonal transport. *Science* 330:198. [CrossRef Medline](#)
- Vossel KA, Xu JC, Fomenko V, Miyamoto T, Suberbielle E, Knox JA, Ho K, Kim DH, Yu GQ, Mucke L (2015) Tau reduction prevents Abeta-induced axonal transport deficits by blocking activation of GSK3beta. *J Cell Biol* 209:419–433. [CrossRef Medline](#)
- Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416:535–539. [CrossRef Medline](#)
- Wolozin B, Davies P (1987) Alzheimer-related neuronal protein A68: specificity and distribution. *Ann Neurol* 22:521–526. [CrossRef Medline](#)
- Wolozin BL, Pruchnicki A, Dickson DW, Davies P (1986) A neuronal antigen in the brains of Alzheimer patients. *Science* 232:648–650. [CrossRef Medline](#)
- Yuan A, Kumar A, Peterhoff C, Duff K, Nixon RA (2008) Axonal transport rates in vivo are unaffected by tau deletion or overexpression in mice. *J Neurosci* 28:1682–1687. [CrossRef Medline](#)